REMARKS

Reconsideration on the merits pursuant to 37 CFR 1.116 is respectfully requested, in light of the amendments and remarks made herein.

New claims 50 and 51 have been added, which further define the nature of the regulatory region that may be used to control transcription of transgenes, and the types of developmental genes amenable to analyses in the claimed transgenic mice, respectively. Support for these claims may be found in the specification at page 17.

Turning now to the Office Action dated July 18, 2001, the rejection of claims 1-49 under 35 U.S.C. §112, first paragraph was maintained, because the specification allegedly fails to enable one skilled in the art to which it pertains to make and/or use the invention. The grounds for the rejection advanced in the Office Action will be addressed individually for the convenience of the Examiner.

First, in response to Applicants' point in the previous Reply that gene activation is not essential to the invention's operability, the Examiner notes that many of the claims require gene expression of the target sequence following recombination. Therefore, while the Examiner agrees that some of the claimed compositions and methods may be used for cell fate mapping and that cell fate mapping may be performed in the absence of gene expression, the Examiner has concluded that the scope of the claims is not congruent with this utility.

Applicants agree that the claims embrace the use of the compositions and methods to activate gene expression, and respectfully submit that such scope is entirely appropriate given the evidence of record. In this regard, Applicants would like to clarify the teachings of the Neuron paper submitted for the Examiner's review in the interview on March 29, 2001, as it appears that such clarification will resolve many of the outstanding issues.

The Examiner is correct in her understanding that the experiments disclosed in the Neuron paper were performed using the same version of FLP as that disclosed in the present

specification. For instance, as discussed in Experimental Procedures, in the section entitled Mice and Genotyping on page 483, it is stated that the Wnt1::FLP transgene was constructed using the F70L version of the FLP recombinase. This is the same FLP used in the present specification (see page 14, line 16, and page 46, line 10, for instance), and the same FLP represented by SEQ ID NO: 17 and specifically recited in claim 17. Moreover, it is clear that the enhanced version of FLP (FLPe) was not used for the experimental system reported in the Neuron paper, particularly given the statement at page 481, col. 2, last paragraph, indicating that work was to be repeated in the future using the enhanced version of FLP. In fact, in the Neuron paper, the FLPe version was only used in double transgenic control mice by constitutively expressing it from a CAG regulatory sequence in order to provide a positive control for double transgenic mice containing Wnt1::FLP and the FRTZ indicator transgene (see Figure 5).

The experiments reported in the Neuron paper fully support the utility and the teachings of the present specification, and show that the materials and methods disclosed in the present specification may be used to achieve FLP-mediated gene activation in transgenic mice. As discussed therein, the authors sought to use the FLP system to examine the fate of certain developing brain regions, and specifically to determine whether both types of precerebellar neurons (mossy fiber axons and climbing fiber axons) are derived from dorsal epithelium progenitors. Thus, by generating mice containing a first transgene comprising the FLP gene expressed developmentally from a dorsal epithelium promoter (Wnt1::FLP), and a second indicator FRTZ transgene expressed from a neural promoter (Hmgcr::FRTZ), one can track whether different neurons passed through the dorsal epithelial line of development by determining which neurons express the recombined version of FRTZ (FRTZ-A). In other words, neurons that are derived from cells that expressed the FLP recombinase during

cellular differentiation will contain a deletion of material between FRT sites and an activation of lacZ reporter gene expression.

Using this system, the authors were able to show that mossy fiber nuclei of the precerebellar system were derived from dorsal epithelial progenitors, but not other precerebellar nuclei. Hence, the authors were able to predict that the other precerebellar neurons (climbing fiber) are derived from a separate progenitor cell population. And significantly, the authors were able to demonstrate these cell fates by screening for activation of Hmgcr::FRTZ reporter gene expression as detected by X-gal histochemistry and immunofluorescence detection of B-galactosidase (see Figure 2 and page 481, sentence spanning columns 1 and 2).

The Examiner indicated in the Office Action on page 7 that it was unclear as to whether targeted integration was used in the Neuron paper, or whether the integration was random as taught in the present specification. The Neuron paper states in the Experimental Procedures section, in the paragraph bridging pages 483-84, that transgenes were injected into fertilized eggs after they were purified away from plasmid sequences. Thus, it is clear that the transgenes were not integrated using a site-specific integration vector, and that the transgenes integrated randomly.

Furthermore, the Neuron paper also discloses a process for prescreening transgene expression in indicator lines of mice, to determine if the particular line will be suitable for cell fate mapping studies, i.e., whether the cells of the mice will express B-galactosidase at a detectable level after exposure to the recombinase (see the paragraph bridging pages 476-77). For instance, a mouse strain was made in which all cells have a recombined copy of Hmgcr::FRTZ, by exposing the indicator construct to the F70L version of FLP constitutively expressed from ACTB regulatory sequences, rather than the developmentally regulated Wnt1 promoter (see also Figure 1D). Thus, it was predetermined whether Hmgcr::FRTZ

expression could be detected in neurons before the authors looked at the developmental effect of Wnt1::FLP expression on the activation of Hmgcr::FRTZ expression.

Applicants also stress that the indicator construct employed in the Neuron study (Hmgcr::FRTZ) used an expression regulatory sequence that was explicitly disclosed in the specification (see page 17, line 2). The FLP constructs used in the study were also disclosed (see page 42, last paragraph, for instance). The Examiner indicated on page 7 of the Office Action that, so long as the Neuron paper used the same methodology as that disclosed in the specification, she agreed that "one could take the teachings of the specification and, using routine experimentation, swap out the promoter used to drive expression of the FLP recombinase and/or the recombination product to obtain the results reported." The above analysis of the Neuron paper confirms that the methodology used therein is the same as that disclosed in the specification. The only difference is that the promoter has been swapped in the indicator construct (recombination product), inserting the Hmgcr (Hmg-CoA reductase) regulatory region. Again, this region was specifically disclosed at page 17, line 2 as being one regulatory region that could be used in the claimed methods. In this regard, Applicants note that new claims 50 and 51 have been added, which encompass the specific regulatory region and developmental genes employed in the Neuron paper.

The clarification of the Neuron reference provided above is supported by the attached declaration by Susan Dymecki, who confirms that the FLP recombinase employed for the Neuron studies was the same as that used for the work reported in the specification. Dr. Dymecki also confirms that the indicator transgene was integrated randomly into the genome in the Neuron study in order to isolate the mice described. Thus, given the above clarification of the teachings of the Neuron paper and the attached §132 declaration, the rejection remaining under §112, first paragraph should be resolved.

Nevertheless, turning to the other grounds offered for the §112, first rejection, the Examiner rejected Applicants comments regarding the precision of the claimed methodology, indicating that no discussion of precision could be found at Example 2, pages 39-40. Applicants respectfully submit that the Examiner apparently overlooked the discussion at page 40, lines 8-9, where it is disclosed that "precise site-specific recombination" was confirmed by sequence analysis of the 0.25 kb amplification product amplified from cells having undergone recombination. Applicants can think of no better way to show that recombination occurs precisely at FRT sites other than to sequence the region of recombination.

The Examiner further points to the results in the specification at pages 40-41 as showing that only partial gene activation can be accomplished given that only 30% of muscle cells were shown to undergo recombination. Applicants respectfully note that the claims do not require "complete" recombination to the extent that complete means every cell. Indeed, in many situations one would not want such "complete" recombination in that the objective may be to observe recombination in only a subset of cells. Furthermore, in individual cells that have undergone recombination, recombination is complete within that cell. Nevertheless, this ground for the rejection appears to be rendered moot in view of the Examiner's acknowledgment on page 7 of the specification that the experiments of the Neuron paper are proof that gene activation may be accomplished and conditions may be optimized using routine experimentation by swapping different regulatory regions.

The Examiner further alleges on page 4 of the Office Action that Applicants have provided no evidence for their assertion that routine screening can be used to identify animals that express detectable levels of ß-galactosidase. Applicants believe that this ground for the rejection should also be resolved by the clarification of the Neuron paper teachings, in that the Neuron paper supports Applicants' arguments that the methodology disclosed in the

specification may be used to identify mice that express adequate levels of marker gene expression following recombination.

For instance, as discussed above, the Neuron paper discloses a process for prescreening transgene expression in indicator lines of mice, to determine if the particular line will be suitable for cell fate mapping studies, i.e., whether the cells of the mice will express B-galactosidase at a detectable level after exposure to the recombinase (see the paragraph bridging pages 476-77). To accomplish the prescreening, a mouse strain was made in which all cells have a recombined copy of Hmgcr::FRTZ, by exposing the indicator construct to the F70L version of FLP constitutively expressed from ACTB regulatory sequences, rather than the developmentally regulated Wnt1 promoter (see also Figure 1D). Thus, it was predetermined whether Hmgcr::FRTZ expression could be detected in neurons before the authors looked at the developmental effect of Wnt1::FLP expression on the activation of Hmgcr::FRTZ expression.

Applicants further note that the Examiner seems to have gotten the impression that the point of insertion of the transgene is critical for the methods claimed. Applicants respectfully emphasize that one need not identify a specific integration site beforehand, because indicator mice can be screened for adequate levels of activity without undue experimentation as taught in the specification at page 45, line 17, and exemplified in the Neuron paper using the claimed methods.

The Examiner rejects Applicants' arguments regarding screening for gene expression, noting that Southern blotting and PCR amplification may be used to detect recombination but not gene activation, and that screening with X-gal was the only method taught in the specification for detecting gene activation (page 5 of Office Action). Applicants respectfully note that they had pointed out in the previous Reply that RT-PCR could be used to detect gene expression. Since RT-PCR amplifies from messenger RNA, such amplification would

in fact detect gene expression. Moreover, the specification also discloses at the paragraph bridging pages 20-21 that in situ hybridization of transcripts may be used, as well as other histochemical reporter genes and fusion proteins. In fact, in situ hybridization of transcripts was successfully used in the Neuron paper to verify FLP expression (see the last paragraph on page 477).

On page 5 of the Office Action, the Examiner rejected Applicants' arguments that activation of gene expression may be accomplished by choosing a transgene with greater biological activity, or a regulatory region that is stronger than that of the human ß-actin gene such as the regulatory region of Hmg-CoA reductase. The Examiner rejected these arguments because she was not sure whether the results reported in the Neuron paper could be used to verify the teachings of the specification, i.e., whether the same FLP recombinase was used. However, the Examiner indicated that if Applicants clarified that the same recombinase and the same methodology was used in the Neuron paper, then she would agree that the results reported therein were supportive of Applicants' arguments. As Applicants have clarified herein that indeed the same recombinase and the same methodology was used, this ground for the rejection has been resolved.

Finally, the Examiner indicates that she agrees that cell lineage marking is a specific utility for the claimed invention, but she awaits further evidence regarding the enablement of this utility. Again, as discussed above, the Neuron paper is probative evidence concerning the enablement of this embodiment. By generating mice containing a first transgene comprising the FLP gene expressed developmentally from a dorsal epithelium promoter (Wnt1::FLP), and a second indicator FRTZ transgene expressed from a neural promoter (Hmgcr::FRTZ), the authors were able to track whether different neurons passed through the dorsal epithelial line of development by determining which neurons express the recombined version of FRTZ (FRTZ-A). In other words, neurons that are derived from a cell lineage that

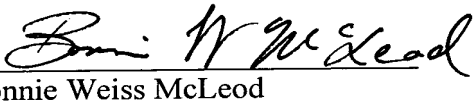
expresses the FLP recombinase during cellular differentiation will contain a deletion of material between FRT sites and an activation of lacZ reporter gene expression. Those neurons that are derived from a different progenitor population will not under go recombination and will not be detectable with X-gal staining. Thus, cell lineage can be determined in this system in relation to a specific progenitor population by either detecting activation of reporter gene expression or by detecting recombination.

In view of all the arguments and evidence presented above, and the declaration under 37 CFR §1.132 attached hereto, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

All issues raised by the Office Action dated July 18, 2001, have been addressed in this Reply. Accordingly, a Notice of Allowance is next in order. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that she contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,

PILLSBURY WINTHROP LLP

By: 

Bonnie Weiss McLeod
Registration No. 43,255

1600 Tysons Boulevard
McLean, Virginia 22102
(703) 905-2000 Telephone
(703) 905-2500 Facsimile

Attorney Reference: 020263-0234805

Date: January 18, 2002